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LIFE HISTORY OF *PUCCINIA SORGHI* SCHW.

by

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A THESIS SUBMITTED FOR THE DEGREE OF

MASTER OF ARTS

UNIVERSITY OF WISCONSIN

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Since 1884 when De Bary suggested a probable sexual act in connection with aecidium many investigators have made a specialized study of different forms of rusts. Special stress has been placed on the formation of the aecidiospores and the relation that rusts bear to higher plants.

Schmitz (1880) four years previous to DeBary in investigating the Uredineae found that the Uredospores and the vegetative cells are binucleated. He considered this analogous to the higher plants.

Massee (1888) studying Uromyces Poae Rab., or Aecidium ranuncularcearum found what he considered to be a rust, which reproduced in a manner very similar to some of the algae. Finding a mass of hyphae in a section of a leaf of Ranunculus Ficaria, he concluded it to be an aecidium of a rust. In other sections he observed the formation of the supposed aecidium. First a small clavate body rose from the center of the mass of hyphae. This body he held was an oogonium. Nearby he found an antheridium which, he believed, fertilized the oogonium. The antheridium became empty and at the same time the oogonium began to increase in size. Then the fertilized oogonium developed nodules, which became cylindrical and cut off a series of spores. These spores when allowed to germinate under water produced zoospores.

Judging from all other observations on the rusts Massee did not observe a rust but some lower fungus or alga.

Rosen (1892) found that all the spores are binucleate and also all the mycelia except that producing aecidiospores. As to the formation of the aecidiospores he concluded that the nucleus of the basal cell divides forming two daughter nuclei. These nuclei separate, one going to the distal portion of the cell, the other remaining where it was formed. The one at the distal portion divides and at the same time a wall is formed cutting off a binucleated aecidiospore. This aecidiospore then cuts off a sterile binucleated cell. The original basal cell continues the process of division until a chain of spores is formed. Every spore cuts off alternately a triangular sterile cell. He concluded that the teleutospore is formed in the same way. First it is a single celled spore with two nuclei. A wall is then formed between the two nuclei, producing a two celled spore, having a single nucleus in each cell. The nucleus in each cell divides, so that the mature teleutospore is a two celled spore and each cell binucleated. Poirault and Raciborski (1895) from their investigations of _Coleosporium Euphrasiae_ concluded that the binucleated condition begins with the sporidium for they found that sometimes the young sporidium contains two nuclei.

They regarded the apparent fusion in the teleutospore as an anaphase and not comparable to a sexual fusion but rather the coming together of the nuclei from the same cytoplasmic mass. They were inclined to think that Schmitz was wrong in his comparison of the binucleated cell in the Uredineae to that of the pollen grain: for the former may be multinucleated while the latter is always (sic) binucleated. Further the nuclei of the pollen grain are sister nuclei while those in Uredineae come from different lines of ancestry.

Sappin-Trouffy (1896) studied Endophyllum Euphorbiae silvaticae. He did very little work on the formation of aecidia as he considered the fusion of nuclei in the teleutospore the important phase in the life cycle of the rust and comparable to the fusion in the ascus. It was in 1893 that Sappin-Trouffy and Dangeard began publishing their cytological researches on the teleutospore. They were the first to observe an actual fusion of the nuclei. As to the spermatia Sappin-Trouffy concluded that they are of a conidial nature.

Richards (1896) studying the aecidium of Uromyces Caladii and of other species, described the formation of the aecidiospore in a somewhat different way than the preceding investigators. He observed that special fertile hyphae mass together. These hyphae can be distinguished

from the others in that they are very granular and stain more deeply. After the massing of the hyphae, the fertile ones bud rapidly and assume a certain position. These form the basal portion of the aecidium. The outer ones become differentiated and form the peridium. He did not agree with Rosen as to the formation of the aecidiospore but thought that they are formed from binucleated hyphae. Rosen had found that after the binucleated spore was cut off each nucleus divides and a small binucleated intercalary cell was formed. Richards observed a different phenomenon. The "twin" nuclei of the spore separate, one staying in the spore and one becoming the nucleus of the intercalary cell, cut off by the spore. The nucleus of the spore always divides once and sometimes three times. He thinks the binucleated condition of the spore is of little significance as he finds it in all other parts of the rusts. He thought that the aecidium should not be regarded as the end of the sexual process but that the spore-bearing hyphae correspond to archicarps.

Juel (1900) studying nuclear behavior in the fusing nuclei of Coleosporium campanulae agreed with Sappin-Trouffy that the binucleated condition of the young basal cell is caused by the blending of two small nuclei. The resulting enlarged nucleus wanders to the center of the cell, where it divides. The newly formed daughter nuclei then divide

by longitudinal spindles. A cross wall then separates the two at the distal portion from those in the center. This structure then becomes the first aecidiospore. In like manner others are cut off, forming a chain of spores.

Maire (1900) investigating Endophyllum Sempervive, var. aeciodes found that aecidiospores develop directly from a uninucleate mycelium. The nucleus of the basal cell enlarges and by simple constriction divides and thereby starts the binuclear condition. He also made a study of the germination of the aecidiospores and found in this species, as Sappin-Trouffy had found, that the aecidiospore develops a promycelium instead of a mycelium. This is brought about in the following manner. The aecidiospore sends out a germinating tube into which passes the nuclei. These divide forming four, each without a nucleole. Cross walls are formed separating the nuclei and from each cell develops a sterigma, on which is borne a sporidium. The nucleus of the cell becomes elongated and is forced through the sterigma into the sporidium, where it develops a nucleole. The nuclei of the sporidia divide amitotically, forming binucleated sporidia. These sporidia develop a mycelium, having two nuclei. They separate and a wall is formed between them producing a uninucleated mycelium.

Arthur (1903), experimenting with Puccinia gramnis Pers. found that direct infection from the aecidia of the Barberry produces a more vigorous state of the rust than an infection by uredospores. The mycelium produced by aecidial infection produces uredospores for only a short time but teleutospores are produced earlier and more abundantly. He concluded, therefore, that the aecidium with the spermogonia represents the sexual stage.

Holden and Harper (1903) examined several species of rust and made a detailed study of Coleosporium sonchi-arvensis. They agree in general with the other authors as to spore formation, but whereas Rosen found sterile cells were cut off alternately, they found in their work that they are cut off irregularly.

As to the development of uredospores they concluded that no cell plate is formed and that the spores are cut off by constriction. This they thought to be very similar to the division in Cladophora. They found the nuclear phenomena of the teleutospore more favorable for study than other stages so a thorough investigation was made of them. They found that the equatorial plate stage is very similar to that found in algae. They observed between six and ten chromosomes, while Sappin-Trouffy and Poirault and Raciborski had contended there are only two chromosomes at this stage. They further advanced the theory that the

fusion of the nuclei in the teleutospore is the only form of sexuality in the life cycle of the rusts. They believed that nuclear rather than cytoplasmic fusion is the essential process of fertilization.

Blackman (1904) was the first to make a definite discovery regarding the fact that there is a sexual fusion in the aecidium. He considered it most reasonable to expect that the fertilization should take place here, as it is the first place where the binucleated stage arises. From his study of Phragmidium violaceum he concluded that a vegetative fusion takes place in the basal cells of the aecidium. The massed hyphae below the epidermis become differentiated into two kinds of cells, fertile cells with small upper sterile cells, which he assumed are reduced trichogynes and vegetative cells. These cells either lie parallel to each other or one below the other. He observed a number of cases in which a nucleus from the vegetative cell was migrating through a pore into the adjoining fertile cell. The pore is often very small so that the nucleus is often reduced to a thread during the process of migration. He was not able to say with certainty whether the cytoplasm migrated with the nucleus or not. The pairing nuclei are always of different sizes, the one of the fertile cell being larger until after completion of fusion when they become equal. This cell, he asserts, must be the result of

a sexual process; the vegetative cell being the male or spermatium and the fertile cell the female. The reason he claims it is a sexual process is that after conjugation the newly formed cell is stimulated to growth and by means of association of nuclei and constriction of walls, cuts off a series of binucleated aecidiospores. The fertilized cell, he described as functioning as the sporophyte generation. Sometimes he found that the nuclei from two vegetative cells migrate into the fertile cell thereby forming a trinucleated cell from which trinucleated spores are formed. This condition is rarely found. He strongly holds to the idea that the ancestors of the rusts were the red algae.

As to the spermatia, Blackman, after a careful study of their structure and habits of germination concluded that they are male cells, which have now become functionless. The nucleus of the vegetative cell replaces it in the process of fertilization in the aecidium.

The uredospores and teleutospores he found to rise from binucleated hyphae.

Christman (1905) in studying Caeoma Nitens and Phragmidium speciosum observed quite a different process from that reported by Blackman. He found that two equal basal cells lying side by side fuse, both cells having an apical degenerate cell. These cells are often found in-

clining towards each other. When they come in contact, the wall dissolves forming a small pore, which becomes larger. The protoplasts fuse and the nuclei come to lie side by side. The nuclei divide by mitosis, two of the daughter nuclei, one from each spindle, wander back to the bases of their respective cells. The other two remain side by side and move to the apical part of the barrel-shaped structure. A cell wall is formed cutting off the first aecidiospore. The remaining nuclei repeat the process until a chain of aecidiospores is formed. The process he regards as a conjugation of equal gametes forming a "non-resting zygospore". He agrees with Blackman that there is reason to believe that the origin of the rusts is from the red algae.

Blackman and Fraser (1906) confirmed Christman's observation on equal cell fusion in Melampsora rostrupi but did not agree with his interpretations of results. They thought it is a case of fertilization by another female cell. In Uromyces Poae and Puccinia poarum many examples of nuclear migration were found. Blackman came, therefore, to the conclusion that there are two types of reduced fertilization which have replaced the normal in absence of spermatia. In one case two fertile female cells conjugate, in the other a conjugation of a female cell with a vegetative cell takes place.

Christman (1907) confirmed his work of 1905 in the

study of Phragmidium potentillae canadensis but thinks that Blackman's idea that the sterile cell is a reduced trichogyne is not very convincing.

Olive (1908) found on examining several species of rusts, Triphragmium ulmarae most favorable for determining the method by which the binucleated condition of the rust arises. He found instances similar to those cited by Christman where equal cells lying side by side fuse, but found that this condition is not universal. Often he found that one of the cells which conjugate lies below the other. He also found instances similar to those observed by Blackman, although he never found the nucleus in migrating so constricted as to appear as a thread. In all cases nuclear migration is followed by cell union, both usually taking place at the same time. He was rather dubious as to the sterile cell lying at the apex of both gametes as described by Christman and thought Blackman was right in his assertion that it occurs on only one of the fusing gametes. He was, however, inclined to agree with Christman that it is of little value in determining the evolution of the rusts. He believed that it is merely a "buffer" cell, produced by the hyphae to break the epidermis. He found that the gametes differ in time of development, which had not been observed by either Blackman or Christman. He came to the final conclusion that Black-

man and Christman were both right as he often found that nuclear migration and the fusing of equal gametes may take place in the same pustule at the same time.

Dittschlag (1910) investigated the cytological structure of _Puccinia Falcariae_. He found that the binucleated condition arises by means of the conjugation of two similar cells. Each fusing cell is well stored with food and has a large nucleus. Sometimes he observed a fusion of three or more cells forming a multinucleated basal cell. The one peculiar feature which all his drawings show is the budding from the basal cell. This has been described to some extent by some of the earlier investigators. These bud-like structures, well shown in Figures 1, 2, 3 and 4, he describes as being the basal cells.

Kurssanow (1910) in restudying the species _Caeoma nitens_ and _Gymnoconia interstitiales_, which had been investigated by Christman and Olive agreed with Christman that only equal cells conjugate and that sterile cells are formed from both the conjugating cells. He thought further that the sterile cells cannot be interpreted as trichogynes but simply as "buffer" cells to break the epidermis. He thought that if a degenerate cell is found on only one of the fusing cells, as Blackman claims, its formation is due to some pathological influences. He agreed with Christman that they are on both cells and are of little consequence in determin-

ing the evolution of rusts. As to the nuclear migration of Blackman he believed that, to be either a pathological phenomenon, or due to fixing. He agreed with Olive that the gametes often differ in time of development.

Olive (1911) in his later paper on "Heteroecism of the Rusts" contended that the aecidium is the primary form of the rust for in this stage we have the uninucleated mycelium, from which by fusion arises the binucleate sporophyte, which later brings about the teleutospore, the other critical stage in the life cycle of the rust.

Taubenhaus (1911) studied the formation and germination of the teleutospores in the species Puccinia Malvacearum. He observed that the teleutospores arise from an unbranched mycelium, the young spore being at first one-celled, later two. The spores produced in late autumn develop with more vigor than those produced earlier. The teleutospore in germinating usually sends out a promycelium from the apical cell although often both cells develop germinating tubes. After the tube has been formed, the contents of the cell flow into it. The tube elongates and often branches into a forked body. The tube often does not mature but grows for a short time then disintegrates. If it develops normally after twelve hours it divides into four cells, then sporidia bud off. These may either develop directly from the cell or bud off from a sterigma. The sporidia when formed are

carried by the wind to a leaf where infection results. The rust might be carried over to the next season in any one of three ways, by the mycelium, by the teleutospore, or by the infected seed.

Plowright (1882) suggested that teleutospores hibernate. Massee (1899) also believed that teleutospores live over winter in debris, yet no experimental evidence was given. Dandeno experimented with teleutospores brought in on the leaves, but they failed to germinate. Taubenhaus, as stated above, was able to secure germination if spores were produced in late autumn. He, however, did not believe that the spores lived over in the embryo but in the bracts and leaves.

Harper (1911) published an article on "Nuclear Phenomena of Sexual Reproduction in Fungi". In discussing the rusts he agreed with Blackman and Christman that in all probability the sexual act occurs in the aecidium instead of the teleutospore. He was of the opinion that since the aecidium is the sporophyte generation, it does not depend on the nuclear fusion for its vigor, and therefore reduction might be accomplished by the mere insertion of a wall between the nuclei. Yet since the nuclei finally fuse in the teleutospore in most species he thought it probably true that there is an interchange of parental qualities here.

Sharp (1911) claimed from his work on <u>Puccinia</u>

Podophylli that a binucleated condition prevails before the aecidium is formed. He regarded the aecidium as a mass of hyphae which arise below the epidermis. He was not clear as to how the formation proceeds, but found that the basal cells contain from two to four nuclei, and the aecidiospores, cut off from these multinucleated cells, correspond to them in the number of nuclei. The binuclear condition is most common. He also held that a similar condition exists in the formation of spermatia. The nuclei divide mitotically and spores are cut off by constriction. The spermatia vary in size and are mostly uninucleate but sometimes binucleate.

Fromme (1912) made a thorough study of the aecidium of Melampsora lini Pers. Desm. The aecidium in this species develops from uninucleated mycelia which travel through the intercellular spaces of the host until they reach the epidermis. Here a massing, interweaving and branching of hyphae take place. The fertile cells push up and are of different shapes due to pressure. Some which are not restricted by epidermis, elongate and at the apex become rather vacuolar. These he concludes are the "buffer" cells described by Christman and are merely for protection. In forming the basal cells he held that two of the vertical cells which are usually in contact conjugate, the wall between, except in the lower part, being absorbed. The area absorbed, he found, differed as Olive also had observed.

He found no normal case of a nuclear migration. He, however, observed that the two gametes do not always lie side by side but often meet at different angles. He observed many instances of fusion. One of his figures (Fig. 11) shows a fusion of every basal cell, so there seems no doubt left as to the origin of the binucleated condition in this species. He also, like some of the previous writers, described triple and quadruple fusion.

Kunkel (1913) studying Caeoma nitens found that the aecidiospores when germinating produce a promycelium, very much like the aecidiospore of Endophyllum Sempervive described by Maire. This promycelium bears sporidia which germinate producing either a second sporidium or a germ tube. For that reason he considered it one of the short cycle rusts.

Kunkel (1914) gave a continuation of his previous paper, describing nuclear behavior in the promycelium. He held that the binucleated aecidiospore previous to germination becomes uninucleated, suggesting therefore a nuclear fusion. The aecidiospore in germination first pushes out a germ tube, into which pass the contents of the spore. The nucleus after making its way into the germ tube elongates and soon divides into two daughter nuclei. This he found is followed by the formation of a cross wall between the nuclei although some times he found that the

four nuclei may be present before cross walls are formed. The germ tube is divided into five cells, the long basal cell, and the four uninucleated cells. From each cell a sterigma develops which bears one or more sporidia. The nucleus elongates and with the cytoplasm passes through the sterigma into the sporidium.

He thought that Caeoma Nitens is a primitive form among the rusts, as it has such a simple form of aecidium. He summed up the life history as follows:

Sporidia produce the uninucleated mycelia, which in turn produce spermogonia and aecidia. He agreed with Christman that the binucleated condition of the aecidiospore arises by the fusion of equal cells in the base of the Caeoma. The aecidiospore then produces the promycelium, which produces sterigmata which bear the sporidia.

Olive (1913) discussed the intermingling of the generations in Puccinia Podophylli, Puccinia obtegens, and Uromyces Glycyrrhizae. In P. Podophylli he found that there is much intermingling of the sporophytic and gametophytic generations. Often in P. Podophylli spore-formation is reversed, so that the teleutospore is produced, followed by uredospores, aecidiospores and spermatia.

Sharp (1911) had observed both uninucleated and binucleated mycelia but failed to explain the condition. Olive found both uninucleate and binucleate mycelia inter-

mingling but found no instances of spermatia budding off from binucleated hyphae or of the spermatia being binucleated. He found instances where the teleutosori arise after inoculation with aecidiospores.

In _P. obtegens_ and _U. Glycyrrhizae_ he found only sporophytic mycelia which produce secondary uredospores and teleutospores in the same sorus. The aecidiospores of _P. Podophylli_ and the uredospores of _P. obtegens_ and _U. Glycyrrhizae_ he regarded as being apogamously formed from the binucleated mycelium.

MATERIALS AND METHODS.

The problem of a cytological and life history study of Puccinia Sorghi Schw. was assigned to me by Professor Jones and Professor Gilbert in the spring of 1913. I am indebted to Mr. E. T. Bartholomew for my first material of the aecidia. In the spring of 1912, he took some plants of Oxalis stricta, which had been sent to Madison from Vermont, and after taking the potted plants to the field, surrounded them by badly rusted (teleuto) corn stalks, of the previous year's growth. The stalks were stuck into the ground and completely surrounded the pots. In June of the same year he found that the leaves of the O. stricta were conspicuously covered with the spermogonia and aecidia. These infected leaves were fixed in Flemming's fluid of various strengths and embedded in paraffin. The embedded material was turned over to me in the spring of 1913. This material was cut 5 to 6μ thick and stained with the triple stain and with iron haemotoxylin. The host tissue was very difficult to stain, but if slides, after being taken out of the bleach were placed for two or three minutes in 95% alcohol to which had been added four to five drops of ammonia the material stained much better. The triple stain gave the best results. During the spring and summer of 1913 repeated inoculations of uredospores were made on the young

corn, infection resulted, and material was prepared for the study of uredospore formation. The teleutospores, which were produced in the green house, were also fixed. It was too late to get much teleutospore material which had wintered over to make inoculations, so this was abandoned until fall. Then abundance of teleutospores were collected from the University Farm.

HISTORY OF CORN RUST.

Puccinia Sorghi Schw. or the corn rust was first known to pathological literature in 1815. It has been known by many different names, such as Uredo Maydis De Candolle (1815), Puccinia Maydis Carradon (1815), Puccinia Sorghi Schweinitz (1831), Puccinia Maydis Bereng (1844), Uredo Zea Desmaz (1840), Puccinia arundinaeceae var. Maydis Cast. (1841) and Puccinia zea Bereng (1845). It still retains the name of Puccinia Sorghi Schw. It is probably a native of America. It is interesting on account of the economic importance of maize. It is widely distributed, being found wherever corn is grown although more abundant in warmer climates. It has not caused much attention, as it does not develop very much until late summer, though in warmer sections it develops earlier and the damage is greater.

The rust affects mostly the leaves and sheaths and

sometimes the tassels. The injury done consists in the destruction of the chlorophyll and the consequent decrease in the formation of carbohydrates.

The uredosori appear first in the summer on the leaves of the young corn plant. The first appearance of the rust is a slight yellowing followed by a deepening of color. As the sorus enlarges it finally breaks the epidermis, forming a linear sorus. The leaves at first show a rather spotted appearance between the veins, later the tips become yellow and the entire leaf is soon devoid of chlorophyll. (Plate I.) Later in the summer and fall, the teleutospores are most prominent. Often they arise from the same sorus and also from distinct sori of their own.

The uredospores are rather large (23μ - 30μ x 22μ - 26μ). The teleutospores are smooth, two celled, and brown, and vary in size (24μ - 43μ X 12μ - 17μ). They are borne on pedicels of medium size.

The heteroecism for this rust was established in 1904 by J. C. Arthur. While on a collecting trip, he noticed aecidia on Oxalis cymosa. Among the debris, near the infected plants, he found some old corn stalks. This suggested to him the possibility of Oxalis being the other host for Puccinia Sorghi. The corn stalks were so covered with silt that he could not detect any rust. The rusted leaves of the Oxalis were taken into the laboratory, where

they were placed so that the spores could fall on the young corn leaves. In a few days the leaves appeared yellow in patches and later uredosori were prominent on the leaves.

This rust seems to be very common in Wisconsin, as the leaves and sheaths of the corn on the University Farm and the nearby fields were very conspicuously covered by the teleutosori in the fall of 1913.

GERMINATION OF TELEUTOSPORES.

On May 5, 1913, some old corn stalks, which were infected by teleutospores were placed about a box of Oxalis but no infection resulted.

Many attempts were made to cause the teleutospores to germinate which had formed in the greenhouse during the spring of 1913. Some were placed in the ice box and then exposed to different degrees of heat but no germination resulted. Others were placed in distilled water and different acids, ether and chloroform, but no method of breaking the rest period was found. On May 22nd 1913 a few teleutosori were found on old corn stalks which had been out in the field over winter. These were placed in tap water. On March 28th a few spores had germinated but the molds attacked them so badly, that the experiment was not carried very far.

Inoculations of the uredospores upon the corn by means

of a suspension were made and in six days uredosori were breaking through the epidermis. Inoculation of the teleutospores on the Oxalis were made in the same manner but with no results. This work was then abandoned until fall, when, October 10th, 1913, corn leaves badly infected by the teleutospores were gathered. Some were placed in the greenhouse while others were tied up in cheese cloth and placed in a cage outside, where they were exposed to the weather.

During the last of February some were brought in and sowed on the water in watch glasses and exposed to different temperatures, but none germinated. The Oxalis was also inoculated at the same time but no infection resulted.

On May 21, 1914 a medium was prepared of 5% beerwort, a weak dung solution, and 1% sugar in which were placed some teleutospores. It was found on March 25th that a few which were on the side of the test tube were germinating, while those down in the medium remained dormant. This suggested that probably the spores needed air for germination. So a number of plaster plates were made. Some of these were saturated with the beerwort medium, others with water, both tap and distilled, and others with a 0.4% sugar solution. The teleutospores were sown on these plates and the following results tabulated:

February 20; placed spores in tap water, no germination.

February 25; subjected spores to different degrees of temperature, no results.

March 21; spores in beerwort medium, germinated March 24th.

March 28; spores sown on plates saturated with beerwort, germinated March 30th.

March 28; spores sown on plates saturated with water, germinated April 3rd.

March 28; spores sown on plates saturated with distilled water, germinated April 3rd.

April 1; spores sown on plates saturated with 0.4% sugar solution, germinated April 2nd.

On March 31st spores were taken from the greenhouse and sown on the plates saturated with the beerwort solution and distilled water. They were germinating on April 2nd although not so well as those which were left outside.

In tap water very few spores germinated and these were weak and of slow growth. Those in the 0.4% sugar solution developed most rapidly, while the greatest number germinated in the beerwort solution and in distilled water.

PROCESS OF GERMINATION.

It was found at the time of germination that the spores become swollen and send out germ tubes from either one or both germ pores. The germ tube is hyaline and contains colorless protoplasm. The tube elongates and divides into four cells (Fig. 10). The basal cell, which is the longest is apparently empty after the septations are formed.

In a number of cases it was found that the teleutospores develop immediately into a mycelium (Fig. 13). It was noticed that this was always true when the spores were submerged but never when they were sown on the plaster plates. This method of germination suggests the probability of the teleutospore infecting the corn directly.

After the formation of the cross walls in the germ tubes, at the apical portion sporidia begin to bud off directly or sometimes sterigmata are developed from which bud off sporidia. The nuclear behavior was not studied. The best temperature for germination seems to be between 60° and 70° Fahr.

The first attempts at inoculation of <u>Oxalis Stricta</u> with teleutospores were made on February 20th, 1914, by means of a suspension, with no results. A second attempt was made on March 25th, when the teleutospores had begun to germinate in the beerwort solution, with no results. The third trial was successful on April 7th. Healthy sori of teleutospores were selected from the leaves and left in distilled water for two hours, then after teasing out the spores they were sprayed on Oxalis. The plant was left in the damp chamber for forty-eight hours. On April 15th well developed spermogonia and aecidia were prominent on several leaves.

SPERMOGONIA AND SPERMATIA.

Seven days after inoculation of Oxalis with teleutospores Spermogonia began to appear. These were fixed at different stages in their development. By studying the prepared and stained sections it was found that the uninucleated mycelium penetrates through the tissue of the host and forms a tangled mesh just beneath the epidermis. The hyphae take on no differential stain but seem to contain a homogeneous substance like water. The spermogonia are formed by fine branches becoming more numerous and inclining towards a point, forming a flask shaped structure, which becomes broader at the base and more deeply seated in the host tissue. The basal portion often extends to the base of the palisade cells and sometimes further. The minute hyphae converge towards a point forming the neck of the flask. Finally the neck breaks through the epidermis, sometimes but apparently not always at a stoma. In the center of the flask and coming from between the hyphae are small elliptical spermatia. These are much smaller than the aecidiospores, although some are well stored with material and have all the appearances of functioning spores. They vary in size. As the spermogonium becomes mature, the neck of the flask becomes broader and the whole structure becomes hemi-spherical in form. The spermatia are then set free.

The formation of the spermagonia usually precedes that of the aecidia but often both are produced at the same time, side by side or on opposite sides of the leaf. The spermagonia are produced on both surfaces of the leaf, as also are the aecidia, although generally the latter are on the under side.

AECIDIA AND AECIDIOSPORES.

A few days after the appearance of the first spermogonia, the young aecidia are apparent. They appear as a series of little cup like structures, arranged in a circle on a swollen base. The material after being fixed, sectioned and stained as described above was carefully examined. It was found that at the beginning of the formation of the aecidium several hyphae mass together and take the form of a sphere. Later the spheroid becomes flattened at the base and a peridium is formed making a flattened receptacle. At the base numerous uninucleated hyphae are massed together and later send up vertical branches. It was of this portion that a thorough study was made.

No fusion is apparent until after the peridium is formed and the massing of the hyphae is complete. The peridium is formed of large binucleated cells, which often remain intact until a great number of spores are formed.

When the aecidium is mature, the peridium breaks almost in the center and turns back, allowing the bright yellow spores to escape.

The formation of the aecidiospore and its place in the life cycle of the rusts has been a field for much research. It has been fully investigated for the Caeoma type of rusts but there is room for much study of those types which have a peridium.

In Puccinia Sorghi it was found that the formation of the binucleated condition in many respects is comparable to what Christman found in Caeoma Nitens and Phragmidium speciosum, Olive in his study of Phragmidium, and Fromme in Melampsora Lini.

Two basal hyphae, either lying side by side or one below the other, come in contact, the wall dissolves and the nuclei migrate to the upper portion of the fused cell. (Fig. 1.) When these nuclei migrate from the basal portion to the apical region of the fusion cell, large vacuoles mark their original position. (Fig. 2.) The greater part of the cytoplasmic contents appear to have migrated also for as seen in (Fig. 2) the lower part of the basal cells appears almost empty.

After the migration mentioned the nuclei divide by longitudinal spindles; one pair of daughter nuclei come to lie in the distal portion, where a constriction is made

preparatory to the cutting off of a binucleated spore (Fig. 3). Christman described the remaining nuclei as wandering back to their respective cells. No evidence of that was seen in this material but always a vacuolar space is observed in the base of each fusing cell.

Fig. 4 represents a preparation in which two cells of different size and different position are fusing. This suggests the fusing of differentiated gametes and the probability of sexual cells, as described by Blackman. There is a remnant of a degenerate cell at the apical portion. This is shown more strongly in Fig. 5 except here it appears as if we have a conjugation of two unequal cells and only one possessing a degenerate cell.

In Fig. 6 is an interesting feature which perhaps shows a step in higher development. Two equal cells have paired, remnants of the old dissolving wall are evident. Each spore formed appears to be perfectly normal. This is characteristic of many of the sections examined, although there are exceptions. In several instances it was found that a perfect spore is cut off, followed by the cutting off of a degenerate cell (Fig. 7).

In a few cases it was found that three cells conjugate and from the basal cell thus formed trinucleated spores are cut off.

THE UREDOSPORE.

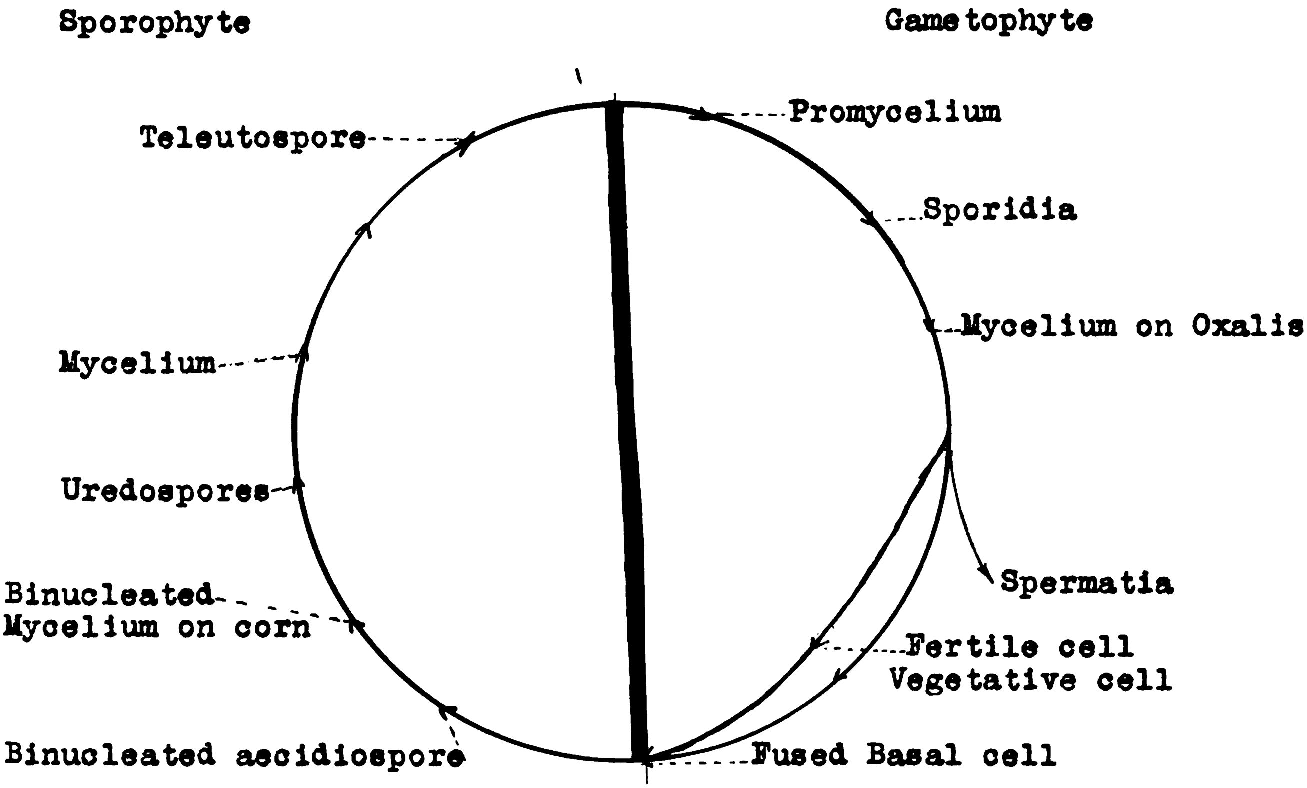
The uredospores are produced in either of two ways; by inoculation of young corn with the uredospores themselves or by inoculation with the aecidiospores. In either case a mycelium is produced which later will develop the uredospores. Five days after infection, light yellow spots begin to appear and two or three days later, the sori are apparent rupturing the epidermis. The uredospore material was fixed, sectioned and stained.

On microscopic examination of this material it was found that the mycelium penetrates into the inner tissue and burrows around in the cells until it finally masses together, forming a spore bed, just under the cuticle. The mycelium is binucleated and at the time of spore formation sends up a series of binucleated hyphae. At first they are nearly of the same thickness throughout and later they become club-shaped. The protoplasm collects in the broad portion and the cells take a spheroidal form, their contents becoming very dense. The nuclei divide and two go to the distal portion. A heavy cross wall is formed and the uredospores, as soon as mature break away from the stalk (Fig. 8).

CONCLUSIONS AND DISCUSSIONS.

1. It is evident from all observations on *Puccinia*

Sorghi that the binucleated condition arises with the fusion of the basal cells of the aecidium. This is a sexual act as has been shown by Blackman, Christman and Olive and in this way arises in the sporophyte generation. The gametophyte begins with the promycelium which produces the sporidia. From the latter is produced a uninucleate mycelium from which arise the functionless spermatia and the fertile and vegetative cells. By the union of these two cells the gametophyte generation ceases and the sporophyte begins. The following diagram illustrates the complete cycle.


Sporophyte
Gametophyte
Teleutospore
Promycelium
Sporidia
Mycelium on Oxalis
Mycelium
Uredospores
Spermatia
Binucleated
Mycelium on corn
Fertile cell
Vegetative cell
Binucleated aecidiospore
Fused Basal cell

The process of fertilization has been fully described for the Caeoma type by Blackman, Christman and Olive. Blackman upheld the nuclear migration theory, Christman the union of two equal cells to form a 'non-resting zygospore', and Olive tried to reconcile the two theories.

From observation of Puccinia Sorghi, it is evident that the aecidiospores of those rusts which possess a peridium are also produced by the fusion of two basal cells. The entire cells appear to conjugate, as was described by Christman but the cells are not always of the same size. Most of the fusing cells are unequal, of different activity and different density (Fig. 2). This supports Blackman's theory of the union of a fertile cell with a vegetative cell.

As to the sterile cells, they are not prominent enough to lead one to any definite conclusion. Their appearance on only a few of the cells suggests the possibility of those rusts with a peridium as being more highly developed in the scale of evolution and the Caeoma type as being more primitive.

Regarding the question of sexual process in the aecidium much has already been said. Although the nuclei do not fuse here, by the union of the basal cells growth is stimulated and reproduction results. The consequence of the beginning of the binucleated condition is the

addition of vigor to the life of the rust.

2. In respect to the germinating of the teleutospores careful experiments have been made and some results obtained.

It is evident that freezing is not necessary for the maturing of the teleutospore, as those which were left in the greenhouse over winter germinated. A rest period is undoubtedly necessary, though this can be shortened to some extent as had been shown by the use of different media.

To secure the formation of sporidia it is necessary for the promycelium to be free to the air. Therefore the plaster plates are the most satisfactory means for securing germination of spores. If grown under the water the spores produce long unicellular branching mycelia. (Fig. 5.) This suggests the possibility, under rare conditions, of producing an infection on the corn by means of the teleutospores.

The corn rust may be carried over the winter by the following different ways: (1) by the teleutospore which rests over winter, then produces a promycelium and sporidia. These infect the Oxalis producing aecidiospores which carry the rust to the corn; (2) in some cases the uredospores which had remained outside were virulent and may again cause infections; (3) the mycelium is perennial and that suggests that if old corn stalks are left in the field, infection

may result from the mycelium.

The producing of aecidia on *Oxalis stricta* by infection of teleutospores confirms the work of E. Bartholomew.

In conclusion, I wish to thank Dr. L. R. Jones and Mr. E. T. Bartholomew for their kind assistance in securing the material and Dr. E. M. Gilbert and Dr. I. F. Lewis for their valuable suggestions and assistance in the cytological work.

BIBLIOGRAPHY.

Arthur, J. C. (1903): The Aecidium as a Device to Restore Vigor to the Fungus. Soc. for Promotion of Agricultural Science.

____________ (1904): Puccinia Sorghi Schw. Bot. Gaz. 38, 64-67.

Blackman, V. H. (1904): Fertilization, Alternation of Generations and General Cytology of the Uredineae. Ann. Bot. 18, 323-73. Pls. 21-24.

Blackman, V. H. and Fraser, Miss H. C. I. (1906): Further Studies on the Sexuality of the Uredineae. Ann. Bot. 20, 35-48. Pls. III & IV.

Christman, A. H. (1905): Sexual Reproduction in the Rusts. Bot. Gaz. 39, 267-74, Pl. VIII.

____________ (1907): The Nature and Development of the Primary Uredospore. Trans. Wis. Acad. Sci., 15, 517-26. Pl. XXIX.

____________ (1907): Alternation of Generations and the Morphology of the Spore Forms in Rusts. Bot. Gaz. 44, 81-101, Pl. VII.

Dittschlag, E. (1910): Zur Kenntnis der Kernverhältnisse von Puccinia Falcariae. Centralbl. f. Bakter. 2, 28, 473, f. 1-6. Pls. I-III.

Dietel, P. (1899) Waren die Rostpilze in frickeren Zeiten plurivor? Bot. Centralbl. 79: 81.

____________ (1895) Ueber Rostpilze mit wiederholter Aecidienbildung. Flora 81, 395.

De Bary, A. (1884): Vergleichende Morphologie u. Biologie der Pilze, u. s. w. Leipzig.

Fromme, F. D. (1912): Sexual Fusions and Spore Development of the Flax Rust. Bull. Torrey Bot. Club, 39, 113-131. Pls. VIII, IX.

Harper, R. A. (1910): Nuclear Phenomena of Sexual Reproduction in Fungi. American Naturalist, 44, 533-546.

Holden, R. J. and Harper R. A. (1903): Nuclear Division and Nuclear Fusion in Coleosporium. Sonchi-arvensis Lev. Transactions of the Wisconsin Academy of Sciences, Arts and Letters, 14, 63-78. Pls. I & II.

Juel, H. O. (1900): Die Kerntheilungen in den Basidien und die Phylogenie der Basidiomyceten. Jahrb. für wiss. Bot. 32, 361-388, Pl. IV.

Kurssanow, L. (1910): Zur Sexualität der Rostpilze. Zeits. Bot. 2:81-93.

Kunkel, L. O. (1913): The Production of a Promycelium by the Aecidiospores of Caeoma Nitens Burrill. Bull. Torrey Bot. Club 40, 361-366.

__________ (1914): Nuclear Behavior in the Promycelium of Caeoma Nitens Burrill and Puc. Pickiana. Howe. American Jour. of Bot. 1:37-45. Pl. III.

Maire, R. (1900): L'evolution Nucleaire Chez les Endophyllum. Jour. de Botanique 14, 80-92, 369-82, Pl. III.

Massee, G. (1888): On the Presence of Sexual Organs in the Aecidium. Ann. Bot. 2, 47-50. Pl. IV.

Olive, E. W. (1911): Origin of Heteroecism in the Rusts. Phytopathology 1, 139-149.

__________ (1908): Sexual Cell Fusions and Vegetative Nuclear Divisions in Rusts. Ann. of Bot. 22, 331-358. Pl. XXII.

__________ (1913): Intermingling of Perennial Sporophytic and Gametophytic Generations in Puccinia Podophylli, Puccinia Obtegens and Uromyces Glycyrrhizae. Annales Mycologici 11, 297-311. P. XV.

Poirault G. and Raciborski, M. (1895): Sur les noyaux des Uredinees. Jour. de Botanique 9, 318-32, 381-88 Pl. VI.

Prichard, F. J. (1911): The Wintering of Puccinia Graminis Tritici E. & H. and the Infection of Wheat through the Seed. Phytopathology 1, 150-54.

Prichard, F. J. (1911): A Preliminary report on the Yearly Origin and Dissemination of Puccinia Graminis. Bot. Gaz. 52, 169-192.

Richards, H. M. (1896): On some Points in the Development of the Aecidia. Proc. Amer. Acad. Arts and Sci. 31, 255-70, P. I.

Rosen, F. (1892): Studien über die Kerne und die Membranbildung bei Myxomyceten und Pilzen. Cohn's Beiträge 4, 237-66.

Sappin-Trouffy, P. (1896): Recherches histologiques sur la famille des Urédinées. Le Botaniste, 5, 59-244.

Sharp, Lester W. (1911): Nuclear Phenomena in Puccinia Podophylli. Bot. Gaz. 51, 463-4.

Taubenhaus, J. J. (1909): A contribution to our Knowledge of the Morphology and Life History of Puccinia Malvacearum Mont. Phytopathology 1, 55, - 62. Pls. XII. XIII. XIV.

EXPLANATION OF PLATE I.

The photograph shows the progressive steps in the Uredo infection.

EXPLANATION OF PLATE II.

The photographs show the healthy leaves of the Oxalis stricta and the infected ones. The upper photograph shows the top surface of the leaves. The healthy leaf is on the right and the infected one is on the leaf and shows the spermogonia and a few aecidial cups. The lower photograph shows the under surface of the same leaves. The aecidial cups are more numerous on the under surface of the infected leaf.

EXPLANATION OF PLATES III, IV, V and VI.

All figures were drawn with the aid of a camera lucida, and with a Leitz objective 1/12 and ocular 12, except drawing of germinating teleutospores, which were made with ocular three and objective 1/12.

Puccinia Sorghi Schw.

Magnification 1275 diameters for teleutospore drawings and 2575 for the other drawings.

Fig. 1. A fusion of two basal cells, one lying below the other. The nuclei are migrating towards the apical portion.

Fig. 2. Nucleus migrating from one cell into the other, leaving a large vacuole in one cell.

Fig. 3. Nuclei dividing by longitudinal spindles, one pair of the daughter nuclei going to the distal portion. A constriction of the wall is shown which precedes the cutting off of the aecidiospore.

Fig. 4. Fusion of two unequal cells and the remnant of a degenerate cell.

Fig. 5. Union of two cells, one possessing a degenerate cell.

Fig. 6. Fusion of two nearly equal cells and production of spores which are normal.

Fig. 7. A basal fusion cell, showing the remnant of the absorbed partition wall. A normal spore and a degenerate spore have been produced by the basal cell.

Fig. 8. The formation of the Uredospores, showing the binucleated hyphae and the vertical club shaped branches which produce the spores.

Fig. 9. A teleutospore sending out a germ tube from each cell.

Fig. 10. A promycelium with a sporidium budding off from the first apical cell and a germ tube developing from the second cell.

Fig. 11. A promycelium with a sterigma forming from the basal cell.

Fig. 12. A promycelium with a long basal cell and sporidia budding off from each cell.

Fig. 13. Teleutospore developing a branching mycelium from the lower cell.

Fig. 14. A promycelium, the three apical cells producing sterigmata on which are borne the sporidia.

PLATE I.

PLATE II.




PLATE III.

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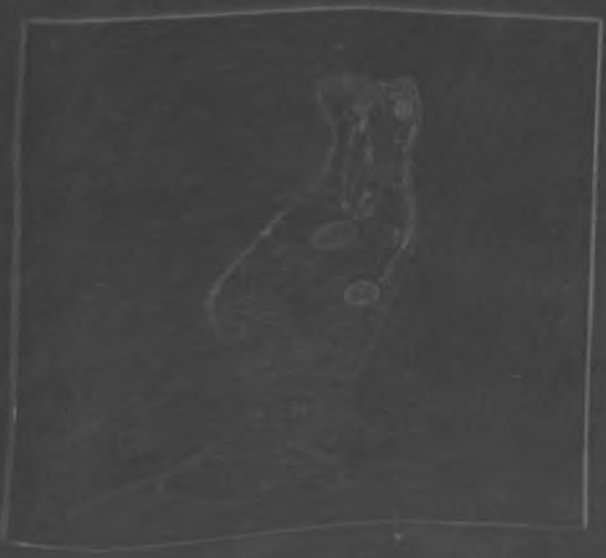

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PLATE IV.

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PLATE V.

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PLATE VI.

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Approved by I. F. Lewis

May 30, 1914.